Phytoestrogens: Epidemiology and a Possible Role in Cancer Protection

Herman Adlercreutz

Department of Clinical Chemistry, University of Helsinki, and Laboratory Department, Helsinki University Central Hospital, Helsinki, Finland

Because many diseases of the Western Hemisphere are hormone-dependent cancers, we have postulated that the Western diet, compared to a vegetarian or semivegetarian diet, may alter hormone production, metabolism, or action at the cellular level by some biochemical mechanisms. Recently, our interest has been mainly focused on the cancer-protective role of some hormonelike diphenolic phytoestrogens of dietary origin, the lignans and the isoflavonoids. The precursors of the biologically active compounds originate in soybean products (mainly isoflavonoids), whole grain cereal food, seeds, and probably berries and nuts (mainly lignans). The plant lignan and isoflavonoid glycosides are converted by intestinal bacteria to hormonelike compounds with weak estrogenic but also antioxidative activity; they have now been shown to influence not only sex hormone metabolism and biological activity but also intracellular enzymes, protein synthesis, growth factor action, malignant cell proliferation, differentiation, and angiogenesis in a way that makes them strong candidates for a role as natural cancer-protective compounds. Epidemiologic investigations strongly support this hypothesis because the highest levels of these compounds in the diet are found in countries or regions with low cancer incidence. This report is a review on recent results suggesting that the diphenolic isoflavonoids and lignans are natural cancer-protective compounds. — Environ Health Perspect 103(Suppl 7):103–112 (1995)

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Introduction

Numerous epidemiologic and migrant studies support the view that the Western diet is one of the main factors causing the high incidence of the so-called Western diseases (1–4), among which we include the major hormone-dependent cancers, colon cancer, and coronary heart disease. Because all these diseases to various extents are related to sex hormones or sex hormone metabolism (5–7), we have postulated that the Western diet, compared to the vegetarian or semivegetarian diet in some developing and Asian countries, may alter hormone production, metabolism, or action at the cellular level by some biochemical mechanisms. Recently, our interest has been focused on the biological role

of two groups of hormonelike diphenolic phytoestrogens of dietary origin, the lignans and the isoflavonoids; they have similar molecular weights and metabolism as the steroids, but partly with clearly different biological effects in the cells. These compounds, which mainly occur in soybean and whole-grain products, various seeds, and other similar food components, have now been shown to influence not only sex hormone metabolism and biological activity but also intracellular enzymes, protein synthesis, growth factor action, malignant cell proliferation, and angiogenesis in a way that makes them strong candidates for a role as cancer-protective compounds. This review discusses those compounds that are measurable in the mammalian organism.

Lignans and Isoflavonoids in Foods

Since 1931 it has been well known that soybeans contain high amounts (up to 100–300 mg/100 g) of the glycosides of the two isoflavones daidzein and genistein (8,9). Much later, a third major compound, glycitein, was found also as a glycoside (10). Small amounts of these three compounds occur in the free form. Fermented soy may contain a catechol conversion product of glycitein, 6,7,4'-trihydroxyisoflavone (11,12). Furthermore, small amounts (about 5 µg/100 g) of the isoflavone coumestrol have been found (13). Soy sauce does

not contain any isoflavones except the lignan precursor coniferyl alcohol (14). Recently, the content of daidzein and genistein glycosides in various soy bean products were determined (15).

We have developed new methodologies for the assay of phytoestrogens in meal, flour, and soy products using gas chromatography–mass spectrometry (GC-MS); the lignan secoisolariciresinol has been measured in soy meal, and the concentrations are relatively low.

The mammalian lignans seem to be mainly derived from grains and seeds and probably also berries and nuts. Our studies on fractionated meals of wheat and rye seem to indicate that the precursors matairesinol and secoisolariciresinol, both present in meal of these grains, are present in the aleurone layer of the grain. This cellular layer is tightly bound to the fiber layer, and the liberation of the precursors from these very resistant cells is difficult.

Phytoestrogens Identified in Man

When consumed, the plant isoflavonoids and lignans undergo many metabolic conversions in the gut, which results in the formation of hormonelike compounds with estrogen activity and the ability to bind weakly to estrogen receptors (16). About 15 years ago, two cyclically occurring unknown compounds, now called enterolactone and enterodiol, were detected in the

urine of the female vervet monkey and in women; these compounds were subsequently identified independently by two groups (*17,18*). Furthermore, small amounts of four plant lignans (matairesinol, lariciresinol, isolariciresinol, and secoisolariciresinol) have been identified in human urine (*19,20*); 7'-hydroxymatairesinol and 7'-hydroxyenterolactone were also tentatively identified in urine (*21*).

The isoflavonoid phytoestrogens are heterocyclic phenols with a close similarity in structure to estrogens and a diphenolic character similar to that of lignans. They occur in numerous plants, and many studies have shown that they have hormonal effects in animals (*22*), the most important being the clover disease. The following isoflavonoid phytoestrogens have been identified or detected in human urine in this laboratory: formononetin, methyl-equol, daidzein, dihydrodaidzein, O-demethylangolensin, genistein, and 3',7-dihydroxyisoflavan. Equol was identified independently in two laboratories (*5,23*). Recently, glycitein was also identified in human urine, and five isoflavonoid metabolites (6'-hydroxy-O-demethylangolensin, dihydrogenistein, dehydro-O-demethylangolensin, and two isomers of tetrahydrodaidzein) were tentatively identified (Figure 1)(*24*).

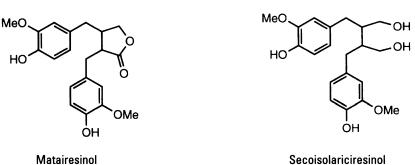## Metabolism of Phytoestrogens in Man

The literature on the origin, formation, and metabolism of the phytoestrogens in animals (*22*) and in man (*16,21,25*) has been reviewed and therefore only a few points will be discussed here.

Equol and O-demethylangolensin are most likely formed, as in sheep, by intestinal bacterial action from formononetin and daidzein present in foodstuffs such as soy products. Some people are unable to produce equol or they excrete this isoflavane in very low amounts [H. Adlercreutz, unpublished results; (*16,26*)]. Of the isoflavonoids, we can now determine daidzein, O-demethylangolensin, equol, and genistein in plasma (*27*), urine (*28*), and feces (*29*) allowing studies on absorption and metabolism of these compounds.

The mammalian lignans enterolactone and enterodiol are formed from plant precursors by the action of intestinal bacteria. The lignan matairesinol is converted to enterolactone and secoisolariciresinol is converted to enterodiol; enterodiol may be oxidized to enterolactone (*16*). We have now established by gas chromatography–mass

Figure 1. Structure of the most important isoflavonoids and lignans identified by human biological samples.

spectroscopy (GC-MS) techniques that the plant lignans are localized close to the outer fiber-containing layers of the grain called the aleurone layer, which contains phytin, polyphenols, enzyme inhibitors, and other compounds generally regarded as antinutritional factors. Modern milling

techniques usually eliminate this fraction because it is tightly bound to the fiber layer, which does not occur in the products supplied to the market for consumption, with some exceptions. This occurs particularly in Western societies. It should be mentioned that administration of

antibiotics almost completely eliminates the formation of enterolactone and enterodiol from plant precursors in the gut (*30,31*). Following further development of our methodology (*28*), we can now analyze matairesinol, secoisolariciresinol, enterodiol, and enterolactone in urine, plasma, and feces. Many of these compounds have also been identified and measured in cow's milk (*32*). Enterolactone concentration is high in human and bovine semen (*33*) and some lignans and isoflavonoids have been identified and measured by GC-MS in saliva, breast aspirate or cyst fluid, and prostatic fluid by GC-MS (*34*).

Phytoestrogen Levels in Various Populations

A summary of our results regarding urinary lignan and isoflavonoid excretion in various dietary groups of women and men, including two groups of breast cancer patients, has recently been presented (*35*). None of the subjects had been treated with antibiotics during the last 3 months.

With regard to lignans, the macrobiotic women living in Boston, Massachusetts, had the highest values, followed by the lactovegetarian women living in Boston and in Helsinki, Finland. The lowest lignan values were found among the breast cancer patients in Boston and in omnivorous women in both Boston and Helsinki. In our study of women in Hawaii that had recently immigrated from the Orient, even lower lignan values were found. These immigrants had similar isoflavonoid values as those consuming an omnivorous Western diet, showing that they had very rapidly left the soy products out of their diet. Leaving the soy from the diet and not consuming any whole-grain bread results in very low lignan and isoflavonoid values in urine, but the low-fat diet may still give some protection against breast cancer. Low lignan values were also found in the Japanese men and women, who had very high isoflavonoid values. Recent measurements of urinary and plasma phytoestrogens in Japanese and Finnish men revealed that despite low urinary lignan values in the Japanese men the values for the free + sulfate-conjugated lignans in plasma were as high as those in the Finnish men, but the glucuronide values for enterolactone were significantly lower ($p < 0.001$) (H Markkanen, unpublished results). In an earlier study we found that lignan excretion in Japanese subjects was related to the intake of whole soybeans (*26*), which

agrees well with our recent detection of secoisolariciresinol in soybean meal.

Biological Effects of Lignans and Isoflavonoids

In this connection only phytoestrogens that have been measured in the human organism will be considered. The lignans enterolactone and enterodiol bind weakly to rat uterine cytosol (JH Clark and H Adlercreutz, unpublished data) but have no detectable estrogenic activity *in vivo* in mice (*30*). However, in four sensitive assays of estrogen activity in tissue culture, including breast cancer cell lines, the lignans were stimulatory and the effect could be blocked by tamoxifen. No antiestrogenic properties could be observed (*36*). In another study enterolactone *in vivo* inhibited estrogen-stimulated RNA synthesis in rat uterine tissue when administered 22 hr before estradiol (*37*). The concentrations of enterolactone were very low, and it is doubtful whether this result can be repeated. We observed stimulatory effect of enterolactone on MCF-7 breast cancer cell growth in the absence of estradiol, but, at slightly stimulatory or nonstimulatory estradiol and physiological enterolactone concentrations, we observed no stimulation or a tendency to inhibition (*38*). Recently enterolactone, but not enterodiol, was shown to stimulate pS2 expression in MCF-7 cells (*39*). These diverging results are difficult to explain, but it has been suggested (*7,40*) that the effect of exogenous weak estrogens may be either agonistic or antagonistic depending on the level of endogenous estrogens; this has been experimentally confirmed with regard to coumestrol (*40*).

Many studies have shown that the isoflavonoid phytoestrogens bind to estrogen receptors and have weak estrogenic activity (*36,41,42*); they also have significant estrogenic effects in animals and in man (*40,43-46*). The most well-known estrogenic effect of phytoestrogens is the clover disease in Australian sheep (*22*). Furthermore, definite antiestrogenic effects have been observed *in vivo* because high levels of synthetic estrogens seem to be counteracted by administered isoflavonoids or their presence in the diet (*43,47,48*).

Several isoflavonoids and lignans compete with estradiol for the rat uterine nuclear type II estrogen binding sites (*49*). The highest affinity with regard to type II site binding of the diphenolic compounds that we found and measured in human urine is shown by the isoflavones daidzein and equol. Also, some lignans like

matairesinol, isolariciresinol, and enterolactone show competition. These binding sites seem to constitute a component of the genome that regulates estrogen-stimulated growth (*50,51*). Thus the antiestrogenic effect of these compounds may be mediated via this binding site.

Many plant lignans have been shown to have anticarcinogenic, antiviral, bactericidic, and fungistatic activities (*16,52,53*). Enterolactone, the most abundant mammalian lignan, is a moderate inhibitor of placental aromatase and competes with the natural substrate androstenedione for the enzyme (*54*). Other experiments with a choriocarcinoma cell line (JEG-3) showed that enterolactone is very readily transferred from cell culture media into the cells (*54*). Flavonoids, occurring in very high amounts in the diet, are inhibitors of the aromatase enzyme (*55*). Studies in human preadipocytes show inhibition of the aromatase enzyme to various degrees by lignans, flavonoids, and isoflavonoids (*56,57*). Most of the lignans and flavonoids are only weak inhibitors. However, a diet rich in vegetables may, due to the abundance of these compounds in the diet, lead to sufficient concentrations (e.g., in fat cells) to reduce conversion of androstenedione to estrone, lowering risk for estrogen-dependent cancer (*58*).

Genistein, an isoflavone that we identified in human urine (*28*) and that occurs in large amounts both in urine and plasma of Japanese subjects consuming a traditional Japanese diet (*26,59*), is a specific inhibitor of tyrosine-specific protein kinases (except the p40 protein–tyrosine kinase), topoisomerase II (*7*), and protein histidine kinase (*60*). Protein–tyrosine kinase activity is associated with cellular receptors for epidermal growth factor, insulin, insulinlike growth factor I, platelet-derived growth factor, and mononuclear phagocyte growth factor. The tyrosine kinases seem to play an important role in cell proliferation and transformation. The enzyme has been associated with oncogene products of the retroviral *src* gene family and is correlated with the ability of retrovirus to transform cells (*61–64*). Tyrosine kinase activity is also associated with breast cancer oncogene expression (*65,66*).

Stimulation of the Synthesis of Sex Hormone-Binding Globulin

Lignans and isoflavonoids seem to stimulate sex hormone-binding globulin (SHBG) synthesis in the liver; in this way, they most

likely reduce the biological effects of sex hormones (67,68). An increase in SHBG results in lowering the percentages of free testosterone and free estradiol and in reducing both the albumin-bound and the free fraction of the sex hormones. This reduces the metabolic clearance rate of the steroids and, in this way, reduces their biological activity. In Finnish women total fiber intake, total fiber intake per kilogram body weight, and grain fiber intake per kilogram body weight correlate positively with urinary excretion of total lignans and isoflavonoids (67,68). The urinary excretion of the two groups of compounds and also enterolactone alone in both premenopausal and postmenopausal Finnish women correlate positively with plasma SHBG and negatively with plasma percentage free estradiol and percentage free testosterone [H Adlercreutz, unpublished results; (67,68)]. In vitro studies using HepG2 liver cancer cells showed that enterolactone (49), genistein (69), and daidzein (M Carson et al., unpublished data) stimulate SHBG synthesis. This seems to explain the higher SHBG values in vegetarians with normal weight (68,70,71).

Lignans, Isoflavonoids, and Cancer

Table 1 shows a number of studies related to possible anticancer effects of isoflavonoids and lignans detected in man. Studies have been mentioned if the pure compounds have been tested or lignans and/or isoflavonoids have been measured. Studies with soy products, with no mention or measurement of isoflavonoids, have not been included.

Subjects with breast cancer or at high risk of breast cancer excrete low amounts of lignans and isoflavonoids (5,68), but subjects living in areas with low risk of hormone-dependent cancers have higher levels (26,31,59,67,68,109). Finnish subjects with medium risk of breast and prostate cancer have relatively high lignan excretion but low isoflavonoid excretion. Japanese subjects at low risk have high isoflavonoid excretion. Interestingly, the biologically active free + sulfate fraction of the lignans is as high in Japanese men as in Finnish men (H Markkanen et al., unpublished results), despite low urinary lignan values and low lignan glucuronide values in plasma. Because in Boston and in Finland the lignan excretion is mainly associated with the intake of grain fiber or whole-grain products [H Adlercreutz, unpublished data; (31,67)], it seems that in the United States

and Finland the risk may, to a relatively high degree, depend on the intake of such products. Higher mean values of lignan in women were observed in North Karelia compared to women in the Helsinki area, which also correlates with breast cancer risk (31). Intake of fruits and berries in Finnish women has a strong correlation with lignan excretion (67). Berries contain the seeds of the plant; these may be rich in lignan precursors.

In addition to possible inhibitory effects on cancer cell proliferation, on production of estrogens from androgens by inhibition of the aromatase enzyme, and on biological activity of sex hormones due to an effect on SHBG synthesis and clearance of these steroids from the circulation, there is evidence suggesting an effect of both lignans and isoflavonoids on the secretion of gonadotrophins and on the length of the menstrual cycle (7,110–112). It is therefore important for us to know which type of fiber and in which form it is consumed.

It is somewhat surprising that recent prospective epidemiological studies do not show any protective effect of fiber (113) with regard to breast cancer risk. However, in this case only the amount of total dietary fiber was determined. Total fiber intake in a subject tells us relatively little about lignan content of the diet and rather little about the effect on the enterohepatic circulation of estrogens affecting plasma estrogen levels (7,114). In agreement with the study of Pryor et al. (115), it has been observed that there is a correlation between fiber intake and menarcheal age in girls (116,117). This may be due to an effect on the enterohepatic circulation of estrogens reducing plasma estrogen levels, to a loss of energy by increased fecal excretion, or to hormonal effects of phytoestrogens associated with the fiber. In addition, the administration of bran to rats postpones menarche (118). Early menarche is associated with increased breast cancer risk (119). Results of studies in postmenopausal women in Boston (71) and in premenopausal women in Helsinki (120) show that the main and, in fact, the only significant difference between the diets of the breast cancer patients and the omnivorous and vegetarian control women was a lower intake of grain products in the breast cancer subjects. If we compare the diets of the Boston and Finnish women, the main difference between them is in the grain and grain-fiber intake, which is much higher in the Helsinki women and they have a lower risk of breast cancer than the Boston

women. This disparity results in differences in lignan excretion. Further, the fat to grain fiber ratio (g/g) seems to be an important additional determinant of the enterohepatic circulation of estrogens (7,114).

In the Finnish women, the significance of the positive correlation between the excretion of lignans and isoflavonoids in urine and plasma SHBG and the negative correlations with percentage free estradiol and percentage free testosterone are stronger than the separate correlations for each group of compounds (67). In two studies we found the lowest SHBG values in breast cancer patients compared to control omnivorous and vegetarian subjects (68,71). In the second part of the Finlandia project dealing with groups of postmenopausal women studied for 1 year, we again found the lowest SHBG values in the breast cancer groups and in omnivorous women and higher values in the vegetarians. There was a significant positive correlation between urinary total diphenol excretion and plasma SHBG (R = 0.64; $p < 0.001$) (49). Plasma SHBG levels are inversely correlated to plasma insulin (121) and androgens. Androgens tend to be high in breast cancer (71) and are likely to play a role in the pathogenesis of breast and prostate cancer.

Our hypothesis with regard to the protective role of phytoestrogens in breast cancer was supported by studies showing that powdered soy bean chips, both before and after denaturation of protease inhibitors, decrease mammary tumor formation in a rat breast cancer model (77). Furthermore, linseed, containing high amounts of lignans, inhibits mammary carcinogenesis in rats (81,82). Genistein, found in human, chimpanzee, and cow urine and in human plasma and feces, is anticarcinogenic, probably due to its inhibitory effect on protein tyrosine kinase (61–64,122) and angiogenesis (98) and perhaps due to its antioxidative properties (97). Genistein and other flavonoids have been shown to be antiproliferative with regard to breast cancer cells (Table 1)(75,88). When fed to rats, soy protein isolates (which in our experience always contain isoflavonoids) inhibit mammary tumor progression (90). Furthermore, epidemiological evidence obtained in Singapore indicates that soy intake protects women for breast cancer (85). Enterolactone alone (0.5–10 µM) stimulates the growth of MCF-7 cells; in the presence of estradiol, both in concentrations that slightly stimulate growth or in

Table 1. Some studies related to possible anticancer effects of isoflavonoids and lignans detected in man.[a]

Type of cancer	Compound or food	Species or cell type	Effect or result	Reference
Breast cancer	Genistein	MCF-7 cells	Competition with estradiol	(42)
Breast cancer	Diet and phytoestrogen excretion	Women	Low urinary excretion in women at higher risk	(5)
Breast cancer	Diet and phytoestrogen excretion	Women	Low urinary excretion in women at higher risk	(31)
Breast cancer	Diet and phytoestrogen excretion	Finnish, American, and Japanese women	Low urinary excretion in women at higher risk Correlation with plasma SHBG	(67)
(VAL 12)Ha-*ras*-transformed cells	Genistein	NIH 3T3 cells	Inhibition of proliferation	(72)
Breast cancer	Diet and phytoestrogen excretion	Women	Lowest urinary excretion in breast cancer	(68)
Prostate cancer	Soy products	Men of Japanese ancestry	Less risk	(73)
Mitogen-induced proliferation	Daidzein	Human lymphocytes	Inhibition of proliferation	(74)
Breast cancer	Daidzein	ZR-75-1 cells	Inhibition	(75)
Erythroleukemia	Genistein	Mouse MEL cells	Induction of differentiation	(76)
Breast cancer	Soy bean chips	Rat	Inhibition of tumor growth	(77)
Melanoma	Genistein	5 cell lines	Induction of differentiation	(78)
Myeloid leukemia	Genistein	Human K562 cells	Induction of differentiation	(79)
Breast cancer	Flaxseed	Rat	Protective	(80,81)
Colon cancer	Flaxseed	Rat	Protective	(80,82,83)
Leukemia	Genistein	Human HL-60, K562 cells	Induction of differentiation	(84)
Breast cancer	Soy food	Women in Singapore	High intake associated with low risk	(85).
Myeloid leukemia	Genistein	ML-1, HL-60 cells	Induction of differentiation	(86)
Myeloid leukemia	Genistein	MO7E cells	Inhibition of proliferation	(87)
Prostatic dysplasia	Soy food	Male mice	Inhibition	(43)
Breast cancer	Genistein, biochanin A	MCF-7 + other cells	Inhibition of proliferation	(88)
Embryonal carcinoma	Genistein	Mouse F9 cells	Induction of differentiation	(89)
Breast cancer	Heated soy bean protein isolate	Rat	Inhibition of tumor progression	(90)
Mitogen-induced proliferation	Plant lignans	Human lymphocytes	Inhibition of proliferation	(91)
Normal cells	Biochanin A	Embryonal hamster cells	Decreased the metabolism of benzo[a]pyrene	(92)
Breast cancer	Enterolactone	MCF-7 cells	Inhibition of proliferation in the presence of estradiol	(38)
Prostatitis	Soy food	Rat	Preventive effect	(93)
Leukemia	Genistein	MOLT-4, HL-60 human cells	Inhibits cell cycle, progression and growth	(94)
Breast cancer	Flaxseed	Rat	Inhibits promotional phase	(82)
Solid pediatric tumors	Genistein	Neuroblastoma, sarcoma	Inhibition of proliferation	(95)
Liver cancer	Enterolactone	HepG2 cells	Stimulation of SHBG synthesis	(49)
Non-P-glycoprotein-mediated multidrug-resistant cells	Genistein	K562/TPA	Reversal of resistance	(96)
Leukemia and TPA-stimulated PMN cells	Genistein	HL-60 and TPA-stimulated PMN cells	Inhibition of hydrogen peroxide formation	(97)
Placental microsomes	Lignans	Human	Inhibition of aromatase	(54)
Endothelial cells	Genistein	Many different endothelial cells	Inhibition of angiogenesis	(98)
Myeloid leukemia	Daidzein	HL-60 cells	Induction of differentiation	(99)
Non-P-glycoprotein-mediated multidrug-resistant cells	Genistein	Many different cell types	Modulation of decreased drug accumulation	(100)
Gastric cancer	Genistein	HGC-27 cells	Growth inhibition	(101)
Monoblastic leukemia	Genistein	U937 cells	Induction of differentiation	(102)
Liver cancer	Genistein	HepG2 cells	Inhibition of proliferation	(69)
Prostate cancer	Genistein	LNCaP, DU-145 cells	Inhibition of proliferation	(103)
Colon cancer	Soy intake	Japanese men, women	Reduced risk	(104)
Gastric, esophagus, colon cancer	Genistein, biochanin A	Many types of cells	Inhibition of proliferation	(105)
Preadipocytes	Biochanin A	Human	Inhibition of aromatase	(56)
TPA-mediated skin tumor	Genistein	Mouse	Inhibition	(106)
Monocytic leukemia	Genistein	Mouse cell line	Cytotoxicity	(107)
Lymphoma	Genistein	Rat Nb2 lymphoma cells	Growth inhibition	(108)

[a]Mainly studies that discuss the role of isoflavonoids in cancer or that use pure isoflavonoids have been included.

lower amounts, the growth was the same or less than the control (*38*). The mechanism of this phenomenon is unknown.

Prostate Cancer

In Japan and some other Asian countries, despite the same incidence of latent and small or noninfiltrative prostatic carcinomas as in the Western countries, the mortality is low (*123–125*). In 1985 after having found very high urinary excretion of isoflavonoids in Japanese men (*126*), we suggested that this could be due to the effect of phytoestrogens, particularly isoflavonoids, inhibiting the growth of the latent cancers (*7,59*). In epidemiological studies, fat and meat show a positive association with prostate cancer mortality, and cereals show a negative association (*4*). Decreased prostate cancer risk has been found in Adventists men (*127*) that have a high consumption of beans, lentils, peas, and some dried fruits (all dietary sources of flavonoids) and in men of Japanese ancestry in Hawaii (*73*) who consume much rice and tofu, a soybean product containing isoflavonoids (*128,129*). We have measured isoflavonoids in 10 different tofu products by GC-MS (*130*), and the daidzein and genistein content varied from 220 to 460 and 580 to 1130 nmole/g, respectively. Mean consumption of soy products (except soy sauce) was 39.2 ± 36.4 g/day in Japanese men; the intake of various soy products in men and women showed a strong positive association with urinary excretion of isoflavonoids (*26*). Lignan excretion in Japanese subjects showed only a positive association with the intake of pulses, beans, and boiled unprocessed soybeans. Preparation of tofu products seems, therefore, to eliminate the lignan precursors from the beans.

Soy has been found to have a protective effect with regard to prostatitis in rats (*93*), but to my knowledge prostatitis has not been associated with prostate cancer. Recently it was found that soy is protective with regard to prostatic dysplasia in a mouse model (*43*). It was also reported that genistein and biochanin A, the precursor of genistein, inhibit the growth of both androgen-dependent and androgen-independent prostate cancer cells in cell cultures (*103*). The well-known therapeutic effect of estrogens in prostate cancer would suggest that phytoestrogens may inhibit prostate cancer-cell growth during the promotional phase of the disease or they may influence differentiation, as shown for genistein, with regard to different types of leukemia and other malignant cells (Table 1). Recently, an epidemiological study of Japanese subjects showed that environmental factors such as diet can substantially impact the likelihood of developing clinically detectable prostate cancer later in life (*131*).

Despite high fat intake, the prostate cancer incidence in Finland, particularly in northeast Finland (*132*), has been much lower than in the United States but higher than in Japan. The higher production of lignans in the gut due to relatively high intake of whole-grain products, particularly rye bread in the low-incidence rural areas in Finland, may perhaps explain this phenomenon. The lignans are weaker estrogens than the isoflavonoids, but a possible protective effect may well be independent from the estrogenic effect.

Thus, epidemiologic studies as well as cell culture and animal experiments provide evidence suggesting that isoflavonoids and perhaps other phytoestrogens like lignans are protective and can lower risk of prostate cancer during the promotional phase of the disease.

Colorectal and Other Cancers

In 1984 I suggested that the lignans may be protective with regard to both breast and colon cancer (*6*). We have observed a higher urinary lignan excretion in subjects consuming a diet that lowers the risk of colon cancer (*133*) or living in areas with low colon cancer risk (*30,132*). Some epidemiologic evidence obtained in Japan (*104*) points to lower colon cancer incidence in areas with high tofu consumption; this is now being further investigated. Five percent linseed (rich in lignans) in the diet of rats seems to protect against colon cancer, (*83*) and genistein and biochanin A inhibit the proliferation of gastric, esophagus, and colon cancer (*101,105*).

Extracts from human urine containing genistein and synthetic genistein have been shown to inhibit the growth of cells from solid pediatric tumors such as neuroblastomas (with both normal and enhanced MYCN oncogene expression), rhabdomyosarcomas, and Ewing's sarcomas (*95*). We showed that such extracts and synthesized genistein inhibited bFGF-stimulated endothelial cell (bovine brain-derived capillary endothelial cells) proliferation and *in vitro* angiogenesis (*98*). Genistein reduced the production of plasminogen activator and plasminogen activator inhibitor-1 (*98*) in cloned bovine microvascular endothelial cells from adrenal cortex.

Lignans and isoflavonoids of dietary origin seem to play a role in the prevention of several types of cancer. By inhibiting the effect of growth factors and angiogenesis, genistein may be more generally an inhibitor of cancer growth. Lignans and isoflavonoids may also be a preventive with regard to some other Western diseases, particularly cardiovascular diseases and osteoporosis, due to the estrogenic and antioxidative effects. The current evidence is not yet sufficient for any dietary recommendations, and further work is needed to establish the role of lignans and isoflavonoids in human health and disease.

REFERENCES

1. Trowell HC, Burkitt DP, eds. Western Diseases: Their Emergence and Prevention. London:Edward Arnold, 1981.
2. Reddy BS, Cohen LA, eds. Diet, Nutrition, and Cancer: A Critical Evaluation. Vol I: Macronutrients and Cancer. Boca Raton, FL:CRC Press, 1986.
3. Reddy BS, Cohen LA, eds. Diet, Nutrition and Cancer: A Critical Evaluation. Vol II: Micronutrients, Nonnutritive Dietary Factors, and Cancer. Boca Raton, FL:CRC Press, 1986.
4. Rose DP, Boyar AP, Wynder EL. International comparison of mortality rates for cancer of the breast, ovary, prostate, and colon, and per capita food consumption. Cancer 58:2363–2371 (1986).
5. Adlercreutz H, Fotsis T, Heikkinen R, Dwyer JT, Woods M, Goldin BR, Gorbach SL. Excretion of the lignans enterolactone and enterodiol and of equol in omnivorous and vegetarian women and in women with breast cancer. Lancet 2:1295–1299 (1982).
6. Adlercreutz H. Does fiber-rich food containing animal lignan precursors protect against both colon and breast cancer? An extension of the "fiber hypothesis." Gastroenterology 86:761–764 (1984).
7. Adlercreutz H. Western diet and Western diseases: some hormonal and biochemical mechanisms and associations. Scand J Clin Lab Invest 50(Suppl 201):3–23 (1990).

8. Walz E. Isoflavon- and saponin-glucoside in *Soja hispida*. Justus Liebigs Annal Chem 498:118–155 (1931).
9. Eldridge A, Kwolek WF. Soybean isoflavones: effect of environment and variety on composition. J Agric Food Chem 31:394–396 (1983).
10. Naim M, Gestetner B, Kirson I, Birk Y, Bondi A. A new isoflavone from soya beans. Phytochemistry 22:237–239 (1973).
11. György P, Murata K, Ikehata H. Antioxidants isolated from fermented soybeans (tempeh). Nature 203:870–872 (1964).
12. Klus K, Borgerpapendore G, Barz W. Formation of 6,7,4'-trihydroxyisoflavone (factor-2) from soybean seed isoflavones by bacteria isolated from tempe. Phytochemistry 34:979–981 (1993).
13. Lookhart GL, Jones BL, Finney KF. Determination of coumestrol in soybeans by high-performance liquid and thin-layer chromatography. Cereal Chem 55:967–972 (1978).
14. Yokotsuka T. Soy sauce biochemistry. Adv Food Res 30:195–329 (1986).
15. Coward L, Barnes NC, Setchell KDR, Barnes S. Genistein, daidzein, and their beta-glycoside conjugates—antitumor isoflavones in soybean foods from American and Asian diets. J Agric Food Chem 41:1961–1967 (1993).
16. Setchell KDR, Adlercreutz H. Mammalian lignans and phytoestrogens. Recent studies on their formation, metabolism and biological role in health and disease. In: Role of the Gut Flora in Toxicity and Cancer (Rowland I, ed). London:Academic Press, 1988;315–345.
17. Stitch SR, Toumba JK, Groen MB, Funke CW, Leemhuis J, Vink J, Woods GF. Excretion, isolation and structure of a phenolic constituent of female urine. Nature 287:738–740 (1980).
18. Setchell KDR, Lawson AM, Mitchell FL, Adlercreutz H, Kirk DN, Axelson M. Lignans in man and in animal species. Nature 287:740–742 (1980).
19. Bannwart C, Adlercreutz H, Fotsis T, Wähälä K, Hase T, Brunow G. Identification of O-desmethylangolensin, a metabolite of daidzein, and of matairesinol, one likely plant precursor of the animal lignan anterolactone, in human urine. Finn Chem Lett 120–125 (1984).
20. Bannwart C, Adlercreutz H, Wähälä K, Brunow G, Hase T. Detection and identification of the plant lignans lariciresinol, isolariciresinol and secoisolariciresinol in human urine. Clin Chim Acta 180:293–302 (1989).
21. Adlercreutz H, Mousavi Y, Loukovaara M, Hämäläinen E. Lignans, isoflavones, sex hormone metabolism and breast cancer. In: The New Biology of Steroid Hormones (Hochberg R, Naftolin F, eds). New York:Raven Press, 1991;145–154.
22. Price KR, Fenwick GR. Naturally occurring oestrogens in foods—a review. Food Addit Contam 2:73–106 (1985).
23. Axelson M, Kirk DN, Farrant RD, Cooley G, Lawson AM, Setchell KDR. The identification of the weak oestrogen equol [7-hydroxy-3-(4'-hydroxyphenyl)chroman] in human urine. Biochem J 201:353–357 (1982).
24. Kelly GE, Nelson C, Waring MA, Joannou GE, Reeder AY. Metabolites of dietary (Soya) isoflavones in human urine. Clin Chim Acta 223:9–22 (1993).
25. Adlercreutz H. Lignans and phytoestrogens. Possible preventive role in cancer. In: Progress in Diet and Nutrition (Horwitz C, Rozen P, eds). Basel:S. Karger, 1988;165–176.
26. Adlercreutz H, Honjo H, Higashi A, Fotsis T, Hämäläinen E, Hasegawa T, Okada H. Urinary excretion of lignans and isoflavonoid phytoestrogens in Japanese men and women consuming traditional Japanese diet. Am J Clin Nutr 54:1093–1100 (1991).
27. Adlercreutz H, Fotsis T, Lampe J, Wähälä K, Mäkelä T, Brunow G, Hase T. Quantitative determination of lignans and isoflavonoids in plasma of omnivorous and vegetarian women by isotope dilution gas-chromatography mass-spectrometry. Scand J Clin Lab Invest 215:5–18 (1993).
28. Adlercreutz H, Fotsis T, Bannwart C, Wähälä K, Brunow G, Hase T. Isotope dilution gas chromatographic-mass spectrometric method for the determination of lignans and

isoflavonoids in human urine, including identification of genistein. Clin Chim Acta 199:263–278 (1991).
29. Adlercreutz H, Fotsis T, Kurzer M, Wähälä K, Mäkelä T, Hase T. Isotope dilution gas chromatographic—mass spectrometric method for the determination of unconjugated lignans and isoflavonoids in human feces, with preliminary results in omnivorous and vegetarian women. Anal Biochem 225:101–108 (1995).
30. Setchell KDR, Lawson AM, Borriello SP, Harkness R, Gordon H, Morgan DML, Kirk DN, Adlercreutz H, Anderson LC, Axelson M. Lignan formation in man—microbial involvement and possible roles in relation to cancer. Lancet 2:4–7 (1981).
31. Adlercreutz H, Fotsis T, Bannwart C, Wähälä K, Mäkelä T, Brunow G, Hase T. Determination of urinary lignans and phytoestrogen metabolites, potential antiestrogens and anticarcinogens, in urine of women on various habitual diets. J Steroid Biochem 25:791–797 (1986).
32. Adlercreutz H, Fotsis T, Bannwart C, Mäkelä T, Wähälä K, Brunow G, Hase T. Assay of lignans and phytoestrogens in urine of women and in cow milk by GC/MS (SIM). In: Advances in Mass Spectrometry-85. Proceedings of the 10th International Mass Spectrometry Conference (Todd JFJ, ed). Chichester, United Kingdom:John Wiley & Sons, 1986;661–662.
33. Dehennin L, Reiffsteck A, Joudet M, Thibier M. Identification and quantitative estimation of a lignan in human and bovine semen. J Reprod Fertil 66:305–309 (1982).
34. Finlay EMH, Wilson DW, Adlercreutz H, Griffiths K. The identification and measurement of 'phyto-oestrogens' in human saliva, plasma, breast aspirate or cyst fluid, and prostatic fluid using gas chromatography-mass spectrometry [abstract]. J Endocrinol 129 (Suppl):49 (1991).
35. Adlercreutz CHT, Goldin BR, Gorbach SL, Höckerstedt KAV, Watanabe S, Hämäläinen EK, Markkanen MH, Mäkelä TH, Wähälä KT, Hase TA, Fotsis T. Soybean phytoestrogen intake and cancer risk. J Nutr 125:757S–770S (1995).
36. Jordan VC, Koch R, Bain RR. Prolactin synthesis by cultured rat pituitary cells: An assay to study estrogens, antiestrogens and their metabolites *in vitro*. In: Estrogens in the Environment. II: Influences on Development (McLachlan JA, ed). New York:Elsevier, 1985;221–234.
37. Waters AP, Knowler JT. Effect of a lignan (HPMF) on RNA synthesis in the rat uterus. J Reprod Fertil 66:379–381 (1982).
38. Mousavi Y, Adlercreutz H. Enterolactone and estradiol inhibit each other's proliferative effect on MCF-7 breast cancer cells in culture. J Steroid Biochem Mol Biol 41:615–619 (1992).
39. Sathyamoorthy N, Wang TTY, Phang JM. Stimulation of pS2 expression by diet-derived compounds. Cancer Res 54:957–961 (1994).
40. Whitten PL, Naftolin F. Dietary estrogens—a biologically active background for estrogen action. In: New Biology of Steroid Hormones (Hochberg RB, Naftolin F, eds). New York:Raven Press, 1991;155–167.
41. Shutt DA, Cox RI. Steroid and phytoestrogen binding to sheep uterine receptors in vitro. J Endocrinol 52:299–310 (1972).
42. Martin PM, Horwitz KB, Ruyan DS, McGuire WL. Phytoestrogen interaction with estrogen receptors in human breast cancer cells. Endocrinology 103:1860–1867 (1978).
43. Mäkelä S, Pylkkänen L, Santti R, Adlercreutz H. Role of plant estrogens in normal and estrogen-related altered growth of the mouse prostate. In: EURO FOOD TOX III. Schwerzenbach, Switzerland:Institute of Toxicology, Swiss Federal Institute of Technology, and University of Zürich, 1991;135–139.
44. Setchell KDR, Gosselin SJ, Welsh MB, Johnston JO, Balistreri WF, Kramer LW, Dresser BL, Tarr MJ. Dietary estrogens—a probable cause of infertility and liver disease in captive cheetahs. Gastroenterology 93:225–233 (1987).
45. Gavaler JS, Galvao-Teles A, Monteiro E, Van Thiel DH, Rosenblum E. Clinical responses to the administration of bourbon phytoestrogens to normal postmenopausal women [abstract]. Hepatology 14:87A (1991).
46. Van Thiel DH, Galvaoteles A, Monteiro E, Rosenblum E,

Gavaler JS. The phytoestrogens present in de-ethanolized bourbon are biologically active—a preliminary study in a postmenopausal woman. Alcohol Clin Exp Res 15:822–823 (1991).

47. Folman Y, Pope GS. The interaction in the immature mouse of potent oestrogens with coumestrol, genistein and other uterovaginotrophic compounds of low potency. J Endocrinol 34:215–225 (1966).

48. Folman Y, Pope GS. Effect of norethisterone acetate, dimethylstilboestrol, genistein and coumestrol on uptake of [^{3}H]oestradiol by uterus, vagina and skeletal muscle of immature mice. J Endocrinol 44:213–218 (1969).

49. Adlercreutz H, Mousavi Y, Clark J, Höckerstedt K, Hämäläinen E, Wähälä K, Mäkelä T, Hase T. Dietary phytoestrogens and cancer: in vitro and in vivo studies. J Steroid Biochem Mol Biol 41:331–337 (1992).

50. Markaverich BM, Clark JH. Two binding sites for estradiol in rat uterine nuclei: relationship to uterotropic response. Endocrinology 105:1458–1462 (1979).

51. Markaverich BM, Upchurch S, Clark JH. Progesterone and dexamethasone antagonism of uterine growth: a role for a second nuclear binding site for estradiol in estrogen action. J Steroid Biochem 14:125–132 (1981).

52. Rao CBS, ed. The Chemistry of Lignans. Waltair, India: Andhra University Press, 1978;377.

53. Ayres DC, Loike JD. Lignans. Chemical, biological and clinical properties. In: Chemistry & Pharmacology of Natural Products (Phillipson JD, Ayres DC, Baxter H, eds). Cambridge, UK: Cambridge University Press, 1990;402.

54. Adlercreutz H, Bannwart C, Wähälä K, Mäkelä T, Brunow G, Hase T, Arosemena PJ, Kellis JT Jr, Vickery LE. Inhibition of human aromatase by mammalian lignans and isoflavonoid phytoestrogens. J Steroid Biochem Mol Biol 44:147–153 (1993).

55. Kellis JT Jr, Vickery LE. Inhibition of human estrogen synthetase (aromatase) by flavones. Science 225:1032–1034 (1984).

56. Campbell DR, Kurzer MS. Flavonoid inhibition of aromatase enzyme activity in human preadipocytes. J Steroid Biochem Mol Biol 46:381–388 (1993).

57. Wang C, Mäkelä T, Hase T, Adlercreutz H, Kurzer MS. Lignans and isoflavonoids inhibit aromatase enzyme in human preadipocytes. J Steroid Biochem Mol Biol 50:205–212 (1994).

58. Henderson BE, Ross R, Bernstein L. Estrogens as a cause of human cancer: the Richard and Hinda Rosenthal foundation award lecture. Cancer Res 48:246–253 (1988).

59. Adlercreutz H, Markkanen H, Watanabe S. Plasma concentrations of phyto-oestrogens in Japanese men. Lancet 342:1209–1210 (1993).

60. Huang JM, Nasr M, Kim YH, Matthews HR. Genistein inhibits protein histidine kinase. J Biol Chem 267:15511–15515 (1992).

61. Akiyama T, Ishida J, Nakagawa S, Ogawara H, Watanabe S-I, Itoh N, Shibuya M, Fukami Y. Genistein, a specific inhibitor of tyrosine-specific protein kinases. J Biol Chem 262:5592–5595 (1987).

62. Markovits J, Linassier C, Fossé P, Couprie J, Pierre J, Jacquemin-Sablon A, Saucier J-M, Le Pecq J-B, Larsen AK. Inhibitory effects of the tyrosine kinase inhibitor genistein on mammalian DNA topoisomerase II. Cancer Res 49:5111–5117 (1989).

63. Ogawara H, Akiyama T, Watanabe S-I, Ito N, Kobori M, Seoda Y. Inhibition of tyrosine protein kinase activity by synthetic isoflavones and flavones. J Antibiot XLII:340–343 (1989).

64. Teraoka H, Ohmura Y, Tsukada K. The nuclear matrix from rat liver is capable of phosphorylating exogenous tyrosine-containing substrates. Biochem Int 18:1203–1210 (1989).

65. Le Cam A. Natural tyrosine-kinase inhibitors. Pathol Biol 39:796–800 (1991).

66. Lehtola L, Lehväslaiho H, Koskinen P, Alitalo K. A chimeric EGFR/neu receptor in functional analysis of the neu oncoprotein. Acta Oncol 31:147–150 (1992).

67. Adlercreutz H, Höckerstedt K, Bannwart C, Bloigu S, Hämäläinen E, Fotsis T, Ollus A. Effect of dietary components, including lignans and phytoestrogens, on enterohepatic circulation and liver metabolism of estrogens, and on sex hormone binding globulin (SHBG). J Steroid Biochem 27:1135–1144 (1987).

68. Adlercreutz H, Höckerstedt K, Bannwart C, Hämäläinen E, Fotsis T, Bloigu S. Association between dietary fiber, urinary excretion of lignans and isoflavonic phytoestrogens, and plasma non-protein bound sex hormones in relation to breast cancer. In: Progress in Cancer Research and Therapy. Vol 35: Hormones and Cancer 3 (Bresciani F, King RTB, Lippman ME, Raynaud JP, eds). New York:Raven Press, 1988;409–412.

69. Mousavi Y, Adlercreutz H. Genistein is an effective stimulator of SHBG production in Hep-G2 human liver cancer cells and suppresses proliferation of these cells in culture. Steroids 58:301–304 (1993).

70. Armstrong BK, Brown JB, Clarke HT, Crooke DK, Hähnel R, Masarei JR, Ratajzak T. Diet and reproductive hormones: a study of vegetarian and nonvegetarian postmenopausal women. J Natl Cancer Inst 67:761–767 (1981).

71. Adlercreutz H, Hämäläinen E, Gorbach SL, Goldin BR, Woods MN, Dwyer JT. Diet and plasma androgens in postmenopausal vegetarian and omnivorous women and postmenopausal women with breast cancer. Am J Clin Nutr 49:433–442 (1989).

72. Okura A, Arakawa H, Oka H, Yoshinari T, Monden Y. Effect of genistein on topoisomerase activity and on the growth of [VAL 12]Ha-ras-transformed NIH 3T3 cells. Biochem Biophys Res Commun 157:183–189 (1988).

73. Severson RK, Nomura AMY, Grove JS, Stemmerman GN. A prospective study of demographics and prostate cancer among men of Japanese ancestry in Hawaii. Cancer Res 49:1857–1860 (1989).

74. Hirano T, Oka K, Kawashima E, Akiba M. Effects of synthetic and naturally occurring flavonoids on mitogen-induced proliferation of human peripheral-blood lymphocytes. Life Sci 45:1407–1441 (1989).

75. Hirano T, Oka K, Akiba M. Antiproliferative effects of synthetic and naturally occurring flavonoids on tumor cells of the human breast carcinoma cell line, ZR-75-1. Res Commun Chem Pathol Pharmacol 64:69–78 (1989).

76. Watanabe T, Shiraishi T, Sasaki H, Oishi M. Inhibitors for protein-tyrosine kinases, ST638 and genistein, induce differentiation of mouse erythroleukemia cells in a synergistic manner. Exp Cell Res 183:335–342 (1989).

77. Barnes S, Grubbs C, Setchell KDR, Carlson J. Soybeans inhibit mammary tumors in models of breast cancer. In: Mutagens and Carcinogens in the Diet (Pariza MW, Aeschbacher HW, Eton JS, Sato S, eds). New York:Wiley-Liss, 1990;239–253.

78. Kiguchi K, Constantinou AI, Huberman E. Genistein-induced cell differentiation and protein-linked DNA strand breakage in human melanoma cells. Cancer Commun 2:271–278 (1990).

79. Honma Y, Okabe-Kado J, Kasukabe T, Hozumi M, Umezawa K. Inhibition of abl oncogene tyrosine kinase induces erythroid differentiation of human myelogenous leukemia K562 cells. Jpn J Cancer Res 81:1132–1136 (1990).

80. Thompson LU, Serraino M. Lignans in flaxseed and breast and colon carcinogenesis. In: Proceedings of the Flax Institute of the United States of America, 25–26 January 1990, Fargo, North Dakota, 1990;30–35.

81. Serraino M, Thompson LU. The effect of flaxseed supplementation on early risk markers for mammary carcinogenesis. Cancer Lett 60:135–142 (1991).

82. Serraino M, Thompson LU. The effect of flaxseed supplementation on the initiation and promotional stages of mammary tumorigenesis. Nutr Cancer 17:153–159 (1992).

83. Serraino M, Thompson LU. Flaxseed supplementation and early markers of colon carcinogenesis. Cancer Lett 63:159–165 (1992).

84. Constantinou A, Kiguchi K, Huberman E. Induction of differentiation and DNA strand breakage in human HL-60 and

K-562 leukemia cells by genistein. Cancer Res 50:2618–2624 (1990).

85. Lee HP, Gourley L, Duffy SW, Estève J, Lee J, Day NE. Dietary effects on breast-cancer risk in Singapore. Lancet 337:1197–1200 (1991).

86. Makishima M, Honma Y, Hozumi M, Sampi K, Hattori M, Umezawa K, Motoyoshi K. Effects of inhibitors of protein tyrosine kinase activity and/or phosphatidylinositol turnover on differentiation of some human myelomonocytic leukemia cells. Leuk Res 15:701–708 (1991).

87. Kuriu A, Ikeda H, Kanakura Y, Griffin JD, Druker B, Yagura H, Kitayama H, Ishikawa J, Nishiura T, Kanayama Y, Yonezawa T, Tarui S. Proliferation of human myeloid leukemia cell line associated with the tyrosine-phosporylation and activation of the proto-oncogene c-kit product. Blood 78:2834–2840 (1991).

88. Peterson G, Barnes S. Genistein inhibition of the growth of human breast cancer cells—independence from estrogen receptors and the multi-drug resistance gene. Biochem Biophys Res Commun 179:661–667 (1991).

89. Kondo K, Tsuneizumi K, Watanabe T, Oishi M. Induction of in vitro differentiation of mouse embryonal carcinoma (F9) cells by inhibitors of topoisomerases. Cancer Res 51:5398–5404 (1991).

90. Hawrylewicz EJ, Huang HH, Blair WH. Dietary soybean isolate and methionine supplementation affect mammary tumor progression in rats. J Nutr 121:1693–1698 (1991).

91. Hirano T, Wakasugi A, Oohara M, Oka K, Sashida Y. Suppression of mitogen-induced proliferation of human peripheral blood lymphocytes by plant lignans. Planta Med 57:331–334 (1991).

92. Chae YH, Coffing SL, Cook VM, Ho DK, Cassady JM, Baird WM. Effects of biochanin A on metabolism, DNA binding and mutagenicity of benzo[a]pyrene in mammalian cell cultures. Carcinogenesis 12:2001–2006 (1991).

93. Sharma OP, Adlercreutz H, Strandberg JD, Zirkin BR, Coffey DS, Ewing LL. Soy of dietary source plays a preventive role against pathogenesis of prostatitis in rats. J Steroid Biochem Mol Biol 43:557–564 (1992).

94. Traganos F, Ardelt B, Halko N, Bruno S, Darzynkiewicz Z. Effects of genistein on the growth and cell cycle progression of normal human lymphocytes and human leukemic MOLT-4 and HL-60 cells. Cancer Res 52:6200–6208 (1992).

95. Schweigerer L, Christeleit K, Fleischmann G, Adlercreutz H, Wähälä K, Hase T, Schwab M, Ludwig R, Fotsis T. Identification in human urine of a natural growth inhibitor for cells derived from solid paediatric tumours. Eur J Clin Invest 22:260–264 (1992).

96. Takeda Y, Nishio K, Morikage T, Kubota N, Kojima A, Kubo S, Fujiwara Y, Niitani H, Saijo N. Reversal of multidrug resistance by genistein in non-P-glycoprotein mediated multidrug-resistant cell line (K562/TPA) [abstract]. Proc Am Assoc Cancer Res 33:476 (1992).

97. Wei HC, Wei LH, Frenkel K, Bowen R, Barnes S. Inhibition of tumor promoter-induced hydrogen peroxide formation in vitro and in vivo by genistein. Nutr Cancer 20:1–12 (1993).

98. Fotsis T, Pepper M, Adlercreutz H, Fleischmann G, Hase T, Montesano R, Schweigerer L. Genistein, a dietary-derived inhibitor of in vitro angiogenesis. Proc Natl Acad Sci USA 90:2690–2694 (1993).

99. Jing YK, Nakaya K, Han R. Differentiation of promyelocytic leukemia cells HL-60 induced by daidzein in vitro and in vivo. Anticancer Res 13:1049–1054 (1993).

100. Versantvoort CHM, Schuurhuis GJ, Pinedo HM, Eekman CA, Kuiper CM, Lankelma J, Broxterman HJ. Genistein modulates the decreased drug accumulation in non-P-glycoprotein mediated multidrug resistant tumour cells. Br J Cancer 68:939–946 (1993).

101. Matsukawa Y, Marui N, Sakai T, Satomi Y, Yoshida M, Matsumoto K, Nishino H, Aoike A. Genistein arrests cell cycle progression at G(2)-M. Cancer Res 53:1328–1331 (1993).

102. Makishima M, Honma Y, Hozumi M, Nagata N, Motoyoshi

K. Differentiation of human monoblastic leukemia u937 cells induced by inhibitors of myosin light chain kinase and prevention of differentiation by granulocyte-macrophage colony-stimulating factor. Biochim Biophys Acta 1176:245–249 (1993).

103. Peterson G, Barnes S. Genistein and Biochanin-A inhibit the growth of human prostate cancer cells but not epidermal growth factor receptor tyrosine autophosphorylation. Prostate 22:335–345 (1993).

104. Watanabe S, Koessel S. Colon cancer: an approach from molecular epidemiology. J Epidemiol 3:47–61 (1993).

105. Yanagihara K, Ito A, Toge T, Numoto M. Antiproliferative effects of isoflavones on human cancer cell lines established from the gastrointestinal tract. Cancer Res 53:5815–5821 (1993).

106. Bowen R, Barnes S, Wei H. Antipromotional effect of the soybean isoflavone genistein [abstract]. Proc Am Assoc Cancer Res 34:555 (1993).

107. Kanatani Y, Kasukabe T, Hozumi M, Motoyoshi K, Nagata N, Honma Y. Genistein exhibits preferential cytotoxicity to a leukemogenic variant but induces differentiation of a non-leukemogenic variant of the mouse monocytic leukemia Mm cell line. Leuk Res 17:847–853 (1993).

108. Buckley AR, Buckley DJ, Gout PW, Liang HQ, Rao YP, Blake MJ. Inhibition by genistein of prolactin-induced nb2 lymphoma cell mitogenesis. Mol Cell Endocrinol 98:17–25 (1993).

109. Adlercreutz H, Hämäläinen E, Gorbach S, Goldin B. Dietary phyto-oestrogens and the menopause in Japan. Lancet 339:1233 (1992).

110. Cassidy A, Bingham S, Carlson J, Setchell KDR. Biological effects of plant estrogens in premenopausal women [abstract]. FASEB J 7(3 Pt II):5000 (1993).

111. Phipps WR, Martini MC, Lampe JW, Slavin JL, Kurzer MS. Effect of flax seed ingestion on the menstrual cycle. J Clin Endocrinol Metab 77:1215–1219 (1993).

112. Orcheson L, Rickard S, Seidl M, Cheung F, Luyengi L, Fong H, Thompson LU. Estrus cycle and organ changes in rats fed flaxseed, its mammalian lignan precursor or tamoxifen [abstract]. FASEB J 7(3 Pt II):1686 (1993).

113. Willett WC, Hunter DJ, Stampfer MJ, Colditz G, Manson JE, Spiegelman D, Rosner B, Hennekens CH, Speizer FE. Dietary fat and fiber in relation to risk of breast cancer. JAMA 21:2037–2044 (1992).

114. Adlercreutz H. Diet and sex hormone metabolism. In: Nutrition, Toxicity, and Cancer (Rowland IR, ed). Boca Raton, FL:CRC Press, 1991;137–195.

115. Pryor M, Slattery ML, Robison LM, Egger M. Adolescent diet and breast cancer in Utah. Cancer Res 49:2161–2167 (1989).

116. Hughes RE. Dietary fibre and female repoductive physiology. In: Dietary Fibre Perspectives—Reviews & Bibliography (Leeds Ar, ed). London:John Libbey & Co., 1990;76–86.

117. de Ridder CM, Thijssen JHH, Van't Veer P, Van Duuren R, Bruning PF, Zonderland ML, Erich WBM. Dietary habits, sexual maturation, and plasma hormones in pubertal girls—a longitudinal study. Am J Clin Nutr 54:805–813 (1991).

118. Arts CJM, Govers CARL, Vandenberg H, Thijssen JHH. Effects of wheat bran and energy restriction on onset of puberty, cell proliferation and development of mammary tissue in female rats. Acta Endocrinol 126:451–459 (1992).

119. Henderson BE, Bernstein L. The international variation in breast cancer rates: an epidemiological assessment. Breast Cancer Res Treat 18:S11–S17 (1991).

120. Adlercreutz H, Fotsis T, Höckerstedt K, Hämäläinen E, Bannwart C, Bloigu S, Valtonen A, Ollus A. Diet and urinary estrogen profile in premenopausal omnivorous and vegetarian women and in premenopausal women with breast cancer. J Steroid Biochem 34:527–530 (1989).

121. Hautanen A, Sarna S, Pelkonen R, Adlercreutz H. Serum sex hormone-binding globulin, cardiovascular risk factors, and adrenal cortisol responses to dexamethasone and corticotropin. Metabolism 42:870–874 (1993).

122. Reddy KB, Mangold GL, Tandon AK, Yoneda T, Mundy GR, Zilberstein A, Osborne CK. Inhibition of breast cancer cell growth *in vitro* by a tyrosine kinase inhibitor. Cancer Res 52:3636–3641 (1992).

123. Ota K, Misu YA. A study on latent carcinoma of the prostate in Japanese. GANN 49(Suppl):283–284 (1958).

124. Breslow NE, Chan CW, Dhom G, Drury RAB, Frnaks LM, Gellei B, Lee YS, Lundberg S, Sparke B, Sternby NH, Tulinius M. Latent carcinoma of prostate at autopsy in seven areas. Int J Cancer 20:680–688 (1977).

125. Yatani R, Chigusa I, Akazaki K, Stemmerman GN, Welsh RA, Correa P. Geographic pathology of latent prostatic cancer. Int J Cancer 29:611–616 (1982).

126. Adlercreutz H, Honjo H, Higashi A, Fotsis T, Hämäläinen E, Hasegawa T, Okada H. Lignan and phytoestrogen excretion in Japanese consuming traditional diet [abstract]. Scand J Clin Lab Invest 48(Suppl 190):190 (1988).

127. Mills PK, Beeson WL, Phillips RL, Fraser GE. Cohort study of diet, lifestyle and prostate cancer in adventist men. Cancer 64:598–604 (1989).

128. Lindner HR. Study of the fate of phyto-oestrogens in the sheep by determination of isoflavones and coumestrol in the plasma and adipose tissue. Aust J Agric Res 18:305–333 (1967).

129. Setchell KDR, Welsh MB. High-performance liquid chromatographic analysis of phytoestrogens in soy protein preparations with ultraviolet, electrochemical and thermospray mass spectrometric detection. J Chromatogr 386:315–323 (1987).

130. Dwyer JT, Goldin BR, Saul N, Gaultieri L, Barakat S, Adlercreutz H. Tofu and soy drinks contain phytoestrogens. J Am Diet Assoc 94:739–743 (1994).

131. Shimizu H, Ross RK, Bernstein L, Yatani R, Henderson BE, Mack TM. Cancers of the prostate and breast among Japanese and white immigrants in Los Angeles County. Br J Cancer 63:963–966 (1991).

132. Teppo L, Pukkala E, Hakama M, Hakulinen A, Herva A, Saxén E. Way of life and cancer incidence in Finland. Scand J Soc Med Suppl 19:1–84 (1980).

133. Korpela JT, Adlercreutz H, Turunen MJ. Fecal free and conjugated bile acids and neutral sterols in vegetarians, omnivores, and in patients with colorectal cancer. Scand J Gastroenterol 23:277–283 (1988).